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Agronomic and structural responses of stockpiled alfalfa–bermudagrass mixtures

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Abstract

The incorporation of alfalfa (*Medicago sativa* L.) into bermudagrass [*Cynodon dactylon* (L.) Pers.] forage systems in the southern United States has increased. Stockpiling this mixture may extend the grazing season into the fall and winter months with high-quality forage. The objective of this 2-year study was to evaluate agronomic and structural responses of alfalfa–bermudagrass mixtures managed under five stockpiling periods (6, 8, 10, 12, or 14 weeks) in two locations (Shorter, AL, and Tifton, GA). Across locations, stockpiling mixtures for 8 weeks or longer (2400 lb DM ac^{-1}, on average) resulted in greater ($P = 0.001$) herbage accumulation than 6 weeks (3185 lb DM ac^{-1}). The alfalfa proportion was similar among stockpiling periods in Shorter but greater ($P = 0.043$) at 10 and 14 weeks than 6, 8, and 12 weeks in Tifton. A location × year × stockpiling interaction was observed for crude protein (CP, $P < 0.001$) and in vitro true dry matter digestibility over 48 h (IVTDMD48, $P < 0.001$). Crude protein concentrations were similar among stockpiling periods in 2020 in both locations. In 2019, however, CP concentrations reduced with increasing stockpiling period length in Shorter and were similar among treatments in Tifton, except for the lesser CP at 8 than at 10, 12, and 14, weeks. Forage IVTDMD48 concentrations declined with increasing stockpiling period length at both locations, with a more pronounced decline in Shorter in 2019. Results suggest that stockpiling alfalfa–bermudagrass mixtures for up to 8 weeks is a viable option to supply high nutritive value forage and lower lodging losses into the early winter months.

Abbreviations: CP, crude protein; DM, dry matter; HA, herbage accumulation; IVTDMD48, in-vitro true dry matter digestibility over 48 h; NV, nutritive value.

1 | INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a cool-season perennial legume with high forage production and nutritive value (NV; Jones & Olsen, 1987; Hakl et al., 2006), which can be grown in monoculture or in mixture with perennial grasses. Incorporating legumes into grass swards, such as bermudagrass [*Cynodon dactylon* (L.) Pers.], can diversify forage mixtures and improve forage NV and soil fertility (Brown & Byrd, 1990; Cantarutti et al., 2002; Beck et al., 2017a). Moreover, nitrogen fixed by the alfalfa component in alfalfa–bermudagrass mixtures decreases the input of inorganic nitrogen fertilizer required by bermudagrass to achieve desired forage production, which helps to reduce production costs and environmental effects (Rouquette & Smith, 2010; Singh et al., 2012; Beck et al., 2017b).

Short days and low temperatures limit the growth of bermudagrass stands (Hendricks et al., 2020). In the late fall, dormancy of warm-season forages is induced, creating a forage production gap until cool-season forages provide adequate forage mass (Hendricks et al., 2020). In mixed swards with alfalfa, alfalfa–bermudagrass may extend the grazing season until late fall with increased proportions of alfalfa, as lower temperatures support alfalfa growth (Brown & Byrd, 1990; Hendricks et al., 2020). In this context, alfalfa–bermudagrass systems may provide an alternative for use as stockpiled forage to extend the grazing season and reduce the need for supplementation.

Stockpiling is the practice of allowing forage to accumulate in a pasture for grazing at a later time when growth is limited (Allen et al., 2011). Stockpiled bermudagrass monocultures can provide forage with moderate-to-high NV (Scarbrough et al., 2001; Bivens et al., 2017; Holland et al., 2018). However, the herbage accumulation (HA) and NV of stockpiled alfalfa–bermudagrass mixtures have not been evaluated. Besides agronomic responses, structural responses, such as canopy height and lodging percentage, can affect HA and forage apprehension by the animal (Santos et al., 2009). We hypothesized that agronomic and structural responses would be negatively affected by increasing stockpiling period length. Therefore, a 2-year evaluation was conducted to assess the agronomic and structural responses of stockpiled alfalfa–bermudagrass mixtures under different stockpiling period lengths.

2 | MATERIAL AND METHODS

2.1 | Experimental location

This 2-year study was conducted in 2019 and 2020 at two locations: E.V. Smith Research Center in Shorter, AL (32.40° N, 85.92° W) and the University of Georgia Tifton Campus Animal Science Farm in Tifton, GA (31.50° N, 83.53° W). Soil at the Shorter location was classified as Marvyn loamy sand (fine-loamy, kaolinitic, thermic Typic Kanhapludult; USDA Soil Survey Staff, 2019). At Tifton, soil was classified as loamy sand soils (fine-loamy, kaolinitic, thermic Plinthic Kandiudults; USDA Soil Survey Staff, 2019). Monthly average of maximum and minimum temperatures and total rainfall for the experimental period were collected from weather stations housed at the experimental site locations and historical averages (100-year average) were collected from the NOAA (NOAA, 2022) and are presented by location (Figure 1).

Core Ideas

- Alfalfa–bermudagrass mixtures stockpiled for 6 weeks had the least herbage accumulation.
- Mixtures stockpiled for 8 weeks provided increased improved nutritive value with least lodging percentage.
- Lodging percentage increased with increased stockpiling period length.
- Forage nutritive value declined with increased stockpiling period length.
- Stockpiling alfalfa–bermudagrass mixtures can extend forage production into the early winter months.

2.2 | Plot establishment and management

Plots were established into dormant bahiagrass using a no-till drill (Tye 2007 Pasture Pleaser, AGCO) planting on 14-inch row spacing in October 2017 at 24 lb ac^{-1} in Shorter, and in February 2018 at 12 lb ac^{-1} in Tifton. Prior to initiation of the current study, alfalfa–bermudagrass mixtures were managed according to a hay production harvest schedule beginning in 2018. In both locations, the first harvest of the stand during the growing season was collected when alfalfa reached 10% bloom and stands maintained a 28-to-35-day harvest schedule thereafter until the initiation of the stockpiling period. In each year, a total of 250 lb potassium (K) ac^{-1} was applied to mixed stands at each location across three application time periods (83 lb K ac^{-1} per period: initiation of harvest season in spring, initiation of stockpiling period, and at the end of the stockpiling season) according to University of Georgia recommendations (Hancock et al., 2015; Kissel and Sonon, 2008).

Prior to initiation of the stockpiling period in each year, plots were harvested to a 3-inch stubble height to remove residual plant biomass. In the first year of the study (2019),

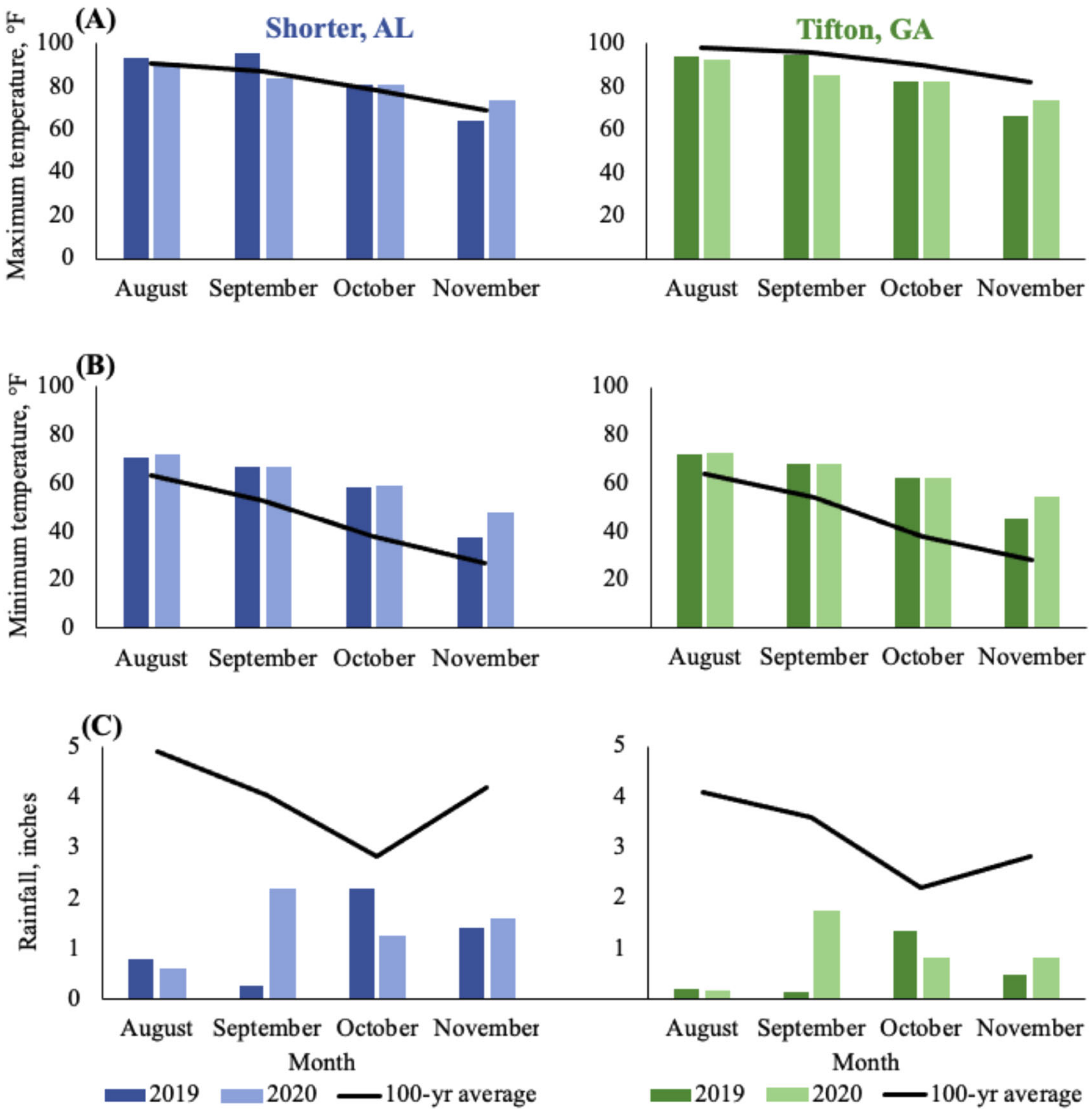

FIGURE 1 Monthly averages of maximum (A) and minimum (B) temperatures and rainfall (C) during the experimental period and historical average (100 year) data at the Edwin V. Smith Research Center, Shorter, AL, and the UGA Tifton Campus Animal Science Farm, Tifton, GA.

TABLE 1 Soil initial values for $pH_{(water)}$ and Mehlich-1 extractable minerals at E.V. Smith Research Center, Shorter, AL, and UGA Tifton Animal Science Farm, Tifton, GA.

Location	$pH_{(water)}$	P	K	Mg	Ca
		lb acre^{-1}			
Shorter	6.4	72	87	179	1294
Tifton	6.8	65	39	138	1169

Note: Ca, Calcium; K, potassium; Mg, magnesium; P, phosphorus.

soil samples were immediately analyzed after residual plant biomass was removed to determine amendments. Soil initial analysis from the 0-to-6-inch depth including $pH_{(water)}$ and Mehlich-1 extractable minerals for both locations are presented in Table 1. Stockpiling initiation and harvest dates are presented in Table 2.

During the experimental period, plots were scouted weekly for potato leafhopper [*Empoasca fabae* (Harris) (Hemiptera: Cicadellidae)], three-cornered alfalfa leafhopper [*Spissistilus festinus* (Say) (Hemipera: Membracidae)], fall armyworm [*Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae)], cowpea aphid [*Aphis craccoivora* (Koch) (Hemiptera: Aphidoidea)], alfalfa weevil [*Hypera postica* (Gyllenhal) (Coleptera: Curculionidae)], and bermudagrass stem maggot [*Atherigona reversua* (Villaneuve) (Dipter: Muscidae)]. Chlorantraniliprole (Prevathon; Corteva Agriscience) was applied at the Tifton location in September 2019 at a rate of 0.112 lb ac^{-1} to control fall armyworms. For control of annual grass weeds, pendimethalin (Prowl H2O, *N*-[1-ethylpropyl]-3,4-dimethyl-2,6-dinitrobenzenamine, BASF Ag Products) was applied at the initiation of the stockpiling period at both locations at a rate of 1.23 lb a.i. ac^{-1}.

TABLE 2 Stockpiling starting date and data collection dates during the experimental period at E.V. Smith Research Center, Shorter, AL, and UGA Tifton Animal Science Farm, Tifton, GA.

Location	Year	Stockpiling period					
		Starting date	6 weeks	8 weeks	10 weeks	12 weeks	14 weeks
Shorter	2019	15 Aug.	26 Sept.	10 Oct.	23 Oct.	5 Nov.	21 Nov.
	2020	10 Aug.	21 Sept.	5 Oct.	19 Oct.	2 Nov.	16 Nov.
Tifton	2019	15 Aug.	26 Sept.	10 Oct.	25 Oct.	8 Nov.	21 Nov.
	2020	13 Aug.	25 Sept.	8 Oct.	22 Oct.	5 Nov.	19 Nov.

2.3 | Treatments and experimental design

Treatments were arranged in a randomized complete block design with four replicates of alfalfa–bermudagrass mixtures at five stockpiling periods (6, 8, 10, 12, or 14 weeks). Each experimental unit (plot) measured 2 ft by 5 ft. Stockpiling treatment plots were comprised of a 1-year-old stand of regionally recommended varieties of alfalfa (Bulldog 805/Alfagraze 600 RR) interseeded into 'Tifton-85' bermudagrass, which was established for a minimum of 10 years prior to the start of this study.

2.4 | Forage response variables

Plots were harvested according to the treatment (stockpiling periods) and the same methods for data collection and measurements were used similarly across both locations. For each sampling event, canopy height was determined within three random 1-ft^2 quadrats within each plot. Nonextended and extended canopy heights were measured using a graded pasture ruler, averaged across all forage material collectively. The lodging percentage was calculated as the percent change in height from the extended to the nonextended height. The nonextended canopy height data are presented and discussed as canopy height.

After canopy heights were measured, the same areas of the three quadrats mentioned above were then harvested to a 4-inch stubble height based on recommendations from Groce (2020) using a cordless grass shear. After sampling, samples were individually hand separated into individual components (alfalfa, bermudagrass, and weed), dried at 140˚F for 72 h, and weighed to determine HA and botanical composition. HA was calculated as the total herbage mass collected within a given stockpiling period. The weed component was considered in the HA calculations. For botanical composition, the 'weed' category was considered as any nonplanted component other than bermudagrass or alfalfa.

After drying and weighed individually for botanical composition, samples were composited by quadrat and then ground to pass a 0.079-inch sieve using a Wiley mill (Thomas-Wiley Laboratory Mill, Thomas Scientific). The samples were then equally split using a sample splitter to create two subsamples, one for wet chemistry and one to be ground to pass through a 0.039-inch sieve (McIntosh et al., 2022) using a Foss Cyclotec sample mill (Foss CT293, Foss Analytical) in preparation for NV analysis via near-infrared reflectance spectroscopy (NIRS).

Forage samples were analyzed for concentrations represented at 100% dry matter (DM) of crude protein (CP), and 48-h in-vitro dry matter digestibility (IVTDMD48) using the appropriate grass hay, mixed hay, and legume hay calibration equations to species treatment, as provided by the NIRS Forage and Feed Testing Consortium (NIRSC, 2022). The calibration statistics for the 2022 NIRSC calibrations are included in Appendix A.

Sample sets by forage product were checked by an initial scan for moisture content which allowed for additional drying of the prepared sample in a forced air oven at 55˚C (McIntosh et al., 2022) This was completed to assure consistent moisture for scanning on a NIRS for less variability in predicted results across all samples (McIntosh et al., 2019; McIntosh et al., 2022). Following this, samples were analyzed using a Foss DS2500 NIR spectrometer (Foss Analytical) that was standardized to the NIRSC master instrument to ensure prediction accuracy. Forage NV data are reported with predictions fitting the allowable $H < 3.0$ (Murray and & Cowe, 2004). For validation of this projects samples a subset of samples (18%) was randomly selected from each harvest for validation of NV parameters. Subsamples were analyzed for CP and IVTDMD48, using the wet chemistry technique for CP (AOAC, 1990) and digestibility (Pomerleau-Lacasse et al., 2018) at Dairy One Forage Testing Laboratory (Ithaca, NY). This lab does not use the same wet chemistry methods used in the NIRSC calibrations; however, it was used with good results for comparison. The standard error of prediction (SEP) for CP was 2.63 and for IVTDMD48 was 3.07, while the coefficient of determination (R^2) for cross validation was 0.69 and 0.91, respectively. Although this validation set had slightly different results due to differing methods, the NIRS predicted output values are compared to the wet chemistry reference values are reasonable considering lab error was not available from the wet chemistry laboratory.

TABLE 3 Location× year × stockpiling period interaction ($P = 0.043$) on herbage accumulation of alfalfa–bermudagrass mixtures at five different stockpiling periods harvested in Shorter, AL, and Tifton, GA.

Location	Year	Stockpiling period					SE	p value
		6 weeks	8 weeks	10 weeks	12 weeks	14 weeks		
		Herbage accumulation, lb DM ac^{-1}						
Shorter	2019	1354cB	2715a	1846bc,yB	1658bcB	2198ab	328	<0.001
	2020	2592cA	3349ab	3820aA	3789aA	2750bc		
Tifton	2019	1598b	2000ab	1913ab	2416a	2344a	328	0.005
	2020	1385b	1597ab	1780ab	2103a	2125a		
Mean	–	1732b	2415a	2340a	2492a	2354a	196	0.001

Note: Means without a common lowercase letter within row are significantly different according to the PDIFF procedure adjusted by Tukey at the 5% level of significance. Means without a common uppercase letter within column within location are significantly different according to the PDIFF procedure adjusted by Tukey at the 5% level of significance.

2.5 | Statistical analysis

Data were analyzed using the PROC GLIMMIX in SAS ver. 9.4 (SAS Institute, 2013). For all data, location (Shorter and Tifton), stockpiling period (6, 8, 10, 12, and 14 weeks), and year (2019 and 2020) were included as fixed effects. Replicate, replicate × location, and replicate × location × year were considered random effects. Means were compared using the PDIFF option adjusted by Tukey's test at the 5% significance level. When location × year × stockpiling period interactions were significant, test of effect slices by location was requested using the SLICE statement.

3 | RESULTS AND DISCUSSION

3.1 | Herbage accumulation and botanical composition

There was a location × year × stockpiling period interaction for HA ($P = 0.043$); therefore, HA is presented by location (Table 3). In Shorter, HA was greater ($P = 0.003$) in 2020 than 2019 (3260 vs. 1954 lb DM ac^{-1}). In 2019, HA increased 100% from 6 to 8 weeks, decreased 39% from 8 to 12 weeks, and when harvest at 14 weeks, was similar to all other stockpiling periods except for being 62% greater than at 6 weeks. In 2020, HA increased 39% from 6 to 8 weeks, remained constant from 8 to 12 weeks, and decreased 27% from 12 to 14 weeks. In Tifton, HA did not differ between years ($P = 0.489$) and averaged 1926 lb DM ac^{-1}. HA was similar among stockpiling periods in both years, except for the lesser ($P = 0.001$) HA for 6 weeks than 12 and 14 weeks (1598 vs. 2416 and 2344 lb DM ac^{-1} in 2019 and 1385 vs. 2103 and 2125 lb DM ac^{-1} in 2020). When pooled across locations and years, there was a significant difference among stockpiling period ($P = 0.001$) on HA with 6 weeks being lesser than all other stockpiling periods (1,732 vs. 2,000 lb DM ac^{-1} on average, respectively).

Environmental conditions, such as temperature and rainfall, play an important role in plant growth and hence HA, with warmer temperatures and increased rainfall stimulating forage growth (Lalman et al., 2000; Coleman et al., 2004; Scarbrough et al., 2004; Patton et al., 2007). Primarily in the southeastern United States, temperatures are historically warmer throughout the summer and early fall and decrease from mid-fall and throughout the winter; while rainfall increases throughout the summer and decreases in early fall (NOAA, 2022; Figure 1). In Shorter in 2019, monthly average maximum temperature in September was higher than the historical average and the other months throughout the experimental period, which may have induced greater forage growth and hence HA at 8 weeks than at 6, 10, and 12 weeks. The lesser HA observed for 6 weeks compared to 8 and 14 weeks may be attributed to reduced days of regrowth and rainfall in August and September. Santos et al. (2022), evaluating stockpiled limpograss [*Hermathria altissima* (Poir.) Stapf & C.E. Hubb.], observed differences in HA between years due to reduced rainfall during the stockpiling period. In 2020 in Shorter, the lesser HA at 6 weeks than at 8, 10, and 12 weeks may be attributed to the significantly reduced rainfall associated with fewer days of regrowth. The lower HA at 14 weeks than at 6, 10, and 12 may be attributed to the rainfall in November being lesser than September (1.58 vs. 2.19 inches) and the historical average (1.58 vs. 4.20 inches) and the average maximum temperature lesser than the previous months, which may have reduced forage growth of the 14-week plots.

In Tifton, the greater HA at 12 and 14 weeks compared to 6 weeks indicates that weather was favorable to support continued forage regrowth following the initial stockpiling period length and avoid weathering deterioration or senescence as 12 and 14 weeks had HA similar to 8 and 10 weeks. Temperatures consistently below freezing (32˚F) and droughty conditions during the stockpiling season decrease herbage mass of stockpiled monoculture bermudagrass over time (Scarbrough et al., 2004). In the present study, the stockpiling periods

TABLE 4 Stockpiling period × location effect on botanical composition of alfalfa–bermudagrass mixtures harvested at five different stockpiling periods harvested in Shorter, AL, and Tifton, GA.

Location	Stockpiling period 6 weeks	8 weeks	10 weeks	12 weeks	14 weeks	SE	*p* value
	Alfalfa, %					7.1	0.043
Shorter	66	57	61	48	51		
Tifton	46b	48b	60a	39b	57a		
	Bermudagrass, %					10.3	0.037
Shorter	30b	41ab	34ab	51a	46ab		
Tifton	32	37	31	33	34		
	Weed, %					3.4	0.006
Shorter	4y	2y	5	1y	3		
Tifton	22abA	15abB	9b	28aA	9b		

Note: Means without a common lowercase letter within row are significantly different according to the PDIFF procedure adjusted by Tukey at the 5% level of significance. Means without a common uppercase letter within column within location are significantly different according to the PDIFF procedure adjusted by Tukey at the 5% level of significance.

evaluated were primarily before the time of a killing frost event and coincide with a mid-to-late fall prospective forage use period. Many previous reports on stockpiled monoculture warm-season grasses target a late fall and early winter grazing period, where weathering effects of standing forage are more prevalent than that observed in this evaluation (Hart et al. 1969; Scarbrough et al., 2004).

There was a stockpiling period × location interaction for proportion of alfalfa ($P = 0.043$), bermudagrass ($P = 0.037$), and weeds ($P = 0.006$) (Table 4). There was no difference among stockpiling periods for proportion of alfalfa in the botanical composition in Shorter, averaging 57%. In Tifton, there was greater proportion of alfalfa on the botanical composition at 14 weeks (57%), compared to 6, 8, and 12 weeks (average 44% across the three periods) stockpiling periods. Bermudagrass proportion was similar among stockpiling periods in Tifton, averaging 34%. In Shorter, 6 weeks had lesser bermudagrass than 12 weeks (30 vs. 51%). Weed proportion, consisting primarily of crabgrass [(*Digitaria sanguinalis* (L.) Scop.], was similar among treatments in Shorter, averaging 3%. In Tifton, 10 and 14 weeks (44%, on average) had lesser weed proportion and 12 weeks (25%).

The lower proportion of alfalfa at the beginning of the stockpiling season in Tifton may be in part explained by its growth distribution and response to high temperature throughout the season. Alfalfa proportion in the mixture is expected to be lower when temperatures are high, which support the rapid growth of warm-season grasses and slowed alfalfa growth, while lower temperatures support rapid alfalfa growth (Brown & Byrd, 1990; Hendricks et al., 2020). Under colder temperatures, Hendricks et al. (2020) reported alfalfa percentages of 30% to up to 60% in alfalfa–bermudagrass mixtures grown in Tifton, GA. The 34% contribution from bermudagrass in the mixture observed in Tifton corroborates with previous studies reporting a 30% to 40% contribution from 'Tifton 85' during the last half of the growing season (Brown & Byrd, 1990; Stringer et al., 1994; Beck et al., 2017a; Hendricks et al., 2020).

3.2 | Canopy height and lodging percentage

There was a stockpiling period × location interaction ($P = 0.012$) on canopy height (Table 5). No difference among stockpiling periods was observed for canopy heights in Tifton, averaging 14 inches. In Shorter, 14 weeks had lower heights than 8 and 10 weeks (11 vs. 15 inches, on average). There was a location × year × stockpiling period interaction ($P = 0.019$) on lodging percentage (Table 6). In Tifton, there was no difference in lodging percentage among stockpiling period or between years, averaging 10% lodging. However, in Shorter, lodging percentage at 14 weeks was greater than 6, 8, and 12 weeks (34 vs. 17%, on average) in 2019. In 2020, lodging percentage increased with an increase in stockpiling period length from 8 to 14 weeks, while 6 weeks had lodging percentage similar to 14 weeks.

The differences in canopy height responses among stockpiling periods in Shorter corroborate with those observed by Wallau et al. (2015) when increasing stockpiling period from 8 to 16 weeks. These authors showed an increase in dead-plant material from 1% to 10% as limpograss stockpiling period increased, which may be due to an overall negative balance between new-plant growth and senescence of plant material and was probably the reason for the responses of canopy height observed in this study. The increase in lodging percentage with increasing stockpiling periods in Shorter also supports data reported by Wallau et al. (2015). These authors reported a linear increase in lodging percentage with

TABLE 5 Stockpiling period × location interaction on canopy height of alfalfa–bermudagrass mixtures harvested at five different stockpiling periods harvested in Shorter, AL, and Tifton, GA.

Location	Stockpiling period					SE	*p* value
	6 weeks	8 weeks	10 weeks	12 weeks	14 weeks		
	Canopy height, inches						
Shorter	14ab	16a	16a	14ab	11b	0.85	0.012
Tifton	15	14	15	13	14		
Mean	15ab	15a	15a	13ab	12b	0.60	0.005

Note: Means without a common letter within row are significantly different according to the PDIFF procedure adjusted by Tukey at the 5% level of significance.

TABLE 6 Location × year × stockpiling period interaction ($P = 0.019$, SE = 4.7) on lodging percentage in alfalfa–bermudagrass mixtures harvested at five different stockpiling periods harvested in Shorter, AL, and Tifton, GA.

Location	Year	Stockpiling period					SE	*p* value
		6 weeks	8 weeks	10 weeks	12 weeks	14 weeks		
		Lodging, %						
Shorter	2019	13bB	17b	32ab	22b	34a	4.7	<0.001
	2020	43abA	22c	30bc	31b	48a		
Tifton	2019	3	3	6	3	11	4.7	0.012
	2020	12	5	18	20	22		
Mean	–	18bc	12c	22b	19b	29a	2.4	<0.001

Note: Means without a common lowercase letter within row are significantly different according to the PDIFF procedure adjusted by Tukey at the 5% level of significance. Means without a common uppercase letter within column within location are significantly different by the PDIFF procedure adjusted by Tukey at the 5% level of significance.

increasing stockpiling period. The differences in lodging percentage among stockpiling periods in Shorter, but not in Tifton, may be in part related to the wetter conditions during the stockpiling period in Shorter and later in the season for both years compared to Tifton. Lodging is related to qualitative and quantitative losses of the canopy and may also limit the ability of the animals to graze the forage material (Santos et al., 2009; Berry et al., 2004; Berry & Spink, 2012). Therefore, Stockpiling periods longer than 12 weeks are not recommended, since they can induce losses due to lodging.

3.3 | Forage nutritive value

The location × year × stockpiling period interaction affected concentrations of CP ($P < 0.001$) and IVTDMD48 ($P < 0.001$) (Table 7). In Shorter, CP reduced with stockpiling periods of 8 weeks or longer in 2019, whereas there was no difference among stockpiling periods in 2020. In Tifton, 8 weeks had CP similar to 6 weeks but lesser than 10, 12, and 14 weeks in 2019, whereas there was no difference among stockpiling periods in 2020. In Shorter, in 2019, IVTDMD48 reduced when mixtures were stockpiled for 8 weeks or longer, with 14 weeks having the least IVTDMD48. In 2020, however, there was no difference for IVTDMD48 among stockpiling periods. In Tifton, IVTDMD48 was reduced when mixtures were stockpiled for 10 weeks in both years.

The negative effect of maturity on forage NV is well established in the literature, where mature stands are greater in fiber and lesser in CP, resulting in reduced digestibility (Burns et al., 1997; Coleman et al., 2004; Mandebvu et al., 1999). Holland et al. (2018) reported a decline in in-vitro DM digestibility (from 68 to 53%) and CP (from 16 to 10%) over time in bermudagrass stands staged in later August and harvested over time from 8 to 20 weeks. The substantially greater NV reported in the current study compared to the values reported for stockpiled monoculture bermudagrass by Holland et al. (2018) is highly related to the presence of alfalfa in this study and to differences in methodologies to analyze forage digestibility. The method for quantification of IVTDMD provides greater concentration than that to quantify in-vitro DM digestibility as reported by Ferreira et al. (2021). In the current study, we report digestibility as IVTDMD, while Holland et al. (2018) report data as in-vitro DM digestibility. Hendricks et al. (2020) evaluated the NV of alfalfa–bermudagrass mixtures harvested at 4 to 5 weeks of regrowth in Tifton, GA. They reported CP concentrations of 12% to 21% and IVTDMD48 levels of 75% to 80% when mixtures were harvested from August to November, with greater CP and IVTDMD48 later in the season when alfalfa

TABLE 7 Location × year × stockpiling period interaction forage nutritive value of alfalfa–bermudagrass mixtures at five different stockpiling periods harvested in Shorter, AL, and Tifton, GA.

Location	Year	Stockpiling period 6 weeks	8 weeks	10 weeks	12 weeks	14 weeks	SE	*p* value
		Crude protein, %					0.8	<0.001
Shorter	2019	26aA	19b	20b	17bc	15c		<0.001
	2020	17y	16	16	17	16		
Tifton	2019	15ab	14bB	17a	17a	16a		<0.001
	2020	16	18x	18	18	18		
Mean	–	19a	17ab	18a	17ab	16b	0.4	0.001
		48-h In-vitro true dry matter digestibility, %					1.1	<0.001
Shorter	2019	86aA	77b	76bA	72bc	69c		<0.001
	2020	73y	74	70y	72	69		
Tifton	2019	72a	71ab	65b	68b	68b		0.001
	2020	73a	75a	65b	66b	63b		
Mean	–	76a	74ab	69c	70bc	67d	0.6	<0.001

Note: Means without a common lowercase letter within row are significantly different according to the PDIFF procedure adjusted by Tukey at the 5% level of significance. Means without a common uppercase letter within column within location are significantly different according to the PDIFF procedure adjusted by Tukey at the 5% level of significance.

proportion in the botanical composition was greater. Thus, the similar CP among stockpiling periods for both years in Tifton is likely associated with the increase in alfalfa proportion later in the stockpiling season, alleviating the negative effect of maturity on the NV of the mixture.

4 | CONCLUSIONS

Results indicate that nutritive value and structural responses can be negatively affected by an increase in stockpiling period. Stockpiling alfalfa–bermudagrass mixtures for 6 weeks results in reduced herbage mass observed across locations, whereas 8 to 14 weeks of stockpiling results in similar HA among stockpiling periods. A stockpiling period of 10 weeks or longer results in reduced nutritive value and increased lodging percentage observed across locations, potentially decreasing the alfalfa–bermudagrass mixture harvesting efficiency. In conclusion, a stockpiling period of 8 weeks may be recommended for alfalfa–bermudagrass mixtures as an alternative to extend the forage production season into the early winter months in the US Southeast. To get more benefits from these mixtures, alfalfa–bermudagrass mixtures should be grazed or harvested in mid-August and grazed early in October, which coincides with late-fall–early-winter prior the onset of killing frost and typically high rainfall in the US Southeast.

AUTHOR CONTRIBUTION

Ana Caroline Vasco: Data curation; Formal analysis; Software; Validation; Visualization; Writing original draft; Writing - reviewing and editing. **Liliane Silva**: Data curation; Formal analysis; Investigation; Supervision; Validation; Visualization; Writing original draft; Writing - reviewing and editing. **Justin Burt**: Data curation; Investigation; Methodology; Supervision; Validation; Visualization; Writing - reviewing and editing. **Katie Mason**: Data curation; Investigation; Methodology; Supervision; Writing - reviewing and editing. **Kim Mullenix**: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing - reviewing and editing. **Chris Prevatt**: Conceptualization; Funding acquisition; Project administration. **Jennifer Tucker**: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing - reviewing and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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APPENDIX A: 2022 Calibration statistics from the NIRS Consortium (Berea, KY) to include the following specific to calibrations used during predictions. Fit is represented by standard error of calibration (SEC), number of samples (N) in calibration data set, r^2, and standard error of cross validation (SECV).

Constituent	*N*	SEC	r^2	SECV
2022 Grass hay calibration				
Crude protein	1179	0.9105	0.9773	0.9586
In-vitro true dry matter digestibility	668	4.1379	0.8457	4.3653
2022 Mixed hay calibration				
Crude protein	1326	0.8164	0.9771	0.8527
In-vitro true dry matter digestibility	406	2.8309	0.8284	3.2715
2022 Legume hay calibration				
Crude protein	954	0.6911	0.9622	0.7462
In-vitro true dry matter digestibility	395	2.6554	0.8559	2.8411